response enhances mitochondrial metabolism triggered mitochondrial hyperpolarization and enhanced ATP production. Li^+ a substrate cation of NCLX but not of NCX replace Na^+ in enhancing the cytosolic and mitochondrial Ca^{2+} responses Altogether, our results show that combined electrical and ion flux activity of TTX sensitive Na^+ channels initiates a cytosolic Na^+ and Ca^{2+} signals propagating by the MCU and NCLX to the mitochondria, thereby shaping cytosolic or mitochondrial Ca^{2+} transients and metabolism of beta cells.

1225-Plat

The Potential for Another Calcium Uptake Mode in Cardiac Mitochondria

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Cytosolic Ca^{2+} levels are tightly regulated in cardiomyoctes. In many instances mitochondria play a direct role in this regulation. They take up Ca^{2+} via the Ca^{2+} uniporter, buffer Ca^{2+} by calcium-phosphate sequestration, and release Ca^{2+} primarily through the electrogenic $Na+/Ca^{2+}$ exchanger. However, the manner in which Ca^{2+} is taken up is still in dispute. In this study we used energized mitochondria isolated from guinea pig hearts to explore the potential for two modes of Ca^{2+} uptake. In addition, we sought to demonstrate the manner in which mitochondria take up, sequester, and release Ca^{2+}, and how Ca^{2+} transport is differentially modulated by Mg^{2+}. To carry out this study, we monitored extra-matrix and matrix [Ca^{2+}] during Ca^{2+} loading and unloading experiments using Fura-4F PP and Fura-4F AM fluorescence to quantify extra-matrix and matrix [Ca^{2+}], respectively. Two loading protocols were used: 1) a bolus of CaCl2 was added to a suspension of mitochondria in respiration buffer and 2) mitochondria were added to respiration buffer already containing CaCl2. In all experiments, ruthenium red was later added to stop Ca^{2+} uptake and NaCl was added to initiate Ca^{2+} efflux. Also, each protocol was executed in the presence and absence of extra-matrix MgCl2. Depending on the protocol, two distinct profiles of Ca^{2+} uptake were observed, whereby using protocol 1 resulted in a faster mode of Ca^{2+} uptake and protocol 2 in a slower mode. Furthermore, Ca^{2+} uptake and efflux were inhibited by MgCl2. We found that Mg^{2+} reduced the ability of mitochondria to sequester Ca^{2+} independent of the protocol. In summary, these observations derived from our experiments show the potential for at least two modes of Ca^{2+} uptake and provide us with a better understanding of how matrix Ca^{2+} dynamics change under physiological and pathophysiological conditions.

1226-Plat

Ultrafast Genetically Encoded Calcium Indicators for Visualizing Calcium Flux and Action Potentials

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Genetically encoded calmodulin-based calcium probes (GCaMPs) have become the reporters of choice for visualising the calcium flux associated with action potentials *in vivo*. A major limitation of currently available GCaMPs is the slow kinetics of fluorescence changes induced by calcium association and dissociation. We have addressed this issue by generating a series of mutants of GCaMP3 in the calcium binding sites of calmodulin alone[1] and in combination with mutations in the RS20 target peptide sequence[2] with the view of lowering the affinity for calcium and accelerating the calcium response kinetics. The calcium association kinetics for the resulting GCaMP3 EF-hand and peptide mutants were highly cooperative and characterized by a rate limiting conformational change. Fluorescence changes on calcium association were up to 7-fold faster compared to GCaMP3. Calcium dissociation rates were up to 60-fold faster than GCaMP3 and 25-fold faster than the newly developed GCaMP6 fast (GCaMP6f). Dissociation constants (K_d) for calcium were in the μM range with Hill coefficients from 2 to 5. Two-photon cross-sections of mutants were comparable to GCaMP3. Fluorescence responses of mutated GCaMP3s to calcium transients in endothelial cells were similar to those seen with small molecule indicators. The principles employed proved to accelerate the calcium kinetics of GCaMP3 and can be applied to the new generations of GCaMPs to generate low affinity probes.

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1227-Plat

A New Ca^{2+} Probe, Calstabi-Cam, Targeted to Ryanodine Receptors of Cardiomyocytes

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The contractile force of cardiomyocytes is controlled by Ca^{2+} cross-signaling between L-type Ca^{2+} channels and ryanodine receptors (RyR2) across the narrow dyadic cleft. To detect the junctional Ca^{2+} signal, we designed a peptide probe (Calstabi-Cam) with calmodulin as its Ca^{2+} sensor, yellow fluorescent protein (EYFP) as reporting fluorophor, and FKBP12.6 (calstabin2) as subunit of RyR2. Effective adenoviral expression in cultured adult rat cardiomyocytes was verified after 48 hours when Calstabi-Cam co-localized with fluorescent RyR antibodies in a sarcomeric z-lines pattern. Dissociation constant (k_d) of Calstabi-Cam for Ca^{2+} measured in permeablized myocytes was 80 nM. The kinetic of Ca^{2+} signals was measured in voltage-clamped cells with a Leica TIRF microscope which allowed comparison of rapidly interlaced images of cytosolic Ca^{2+} probes (fura-2 or fluo-4) and Calstabi-Cam. Fluo-4 Ca^{2+} sparks were detected superimposed on the sarcomeric fluorescence patterns of Calstabi-Cam. On activation of Ca^{2+} release by caffeine or membrane depolarization, Calstabi-Cam fluorescence signals had slower rise times compared to fura-2, but had much slower decay kinetics. Scans of focal Calstabi-Cam signals at different sites, occurring spontaneously or at the onset of evoked Ca^{2+} releases, appeared to have a significant distribution of magnitudes and latencies. We conclude that Ca^{2+}-sensing biological peptides may be targeted to the cleft-space occupied by DHPR/RyRs complex as to make it possible to record the variance of Ca^{2+} signals at different dyadic junctions.

1228-Plat

CaMKII-Mediated Amplification is Essential to NAADP Signalling in Cardiac Myocytes

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NAADP is a highly potent endogenous Ca^{2+}-mobilising second-messenger forming part of the beta-adrenergic response in cardiac myocytes[12]. Our previous work suggests NAADP causes Ca^{2+}-release from acidic endolysosomal stores, leading to additional uptake by the SR[12]. Questions have arisen regarding whether the magnitude NAADP-mediated responses can be accounted for by acidic-store-mediated Ca^{2+} release through TPC channels[34]. This study aimed to confirm that TPC2 channels are required for NAADP responses in cardiac myocytes and investigate the possibility of amplification in the pathway.

Transgenic mice were utilised to investigate the role of TPCs. Rapid application of NAADP-AM to WT murine ventricular myocytes elicited a significant increase in calcium transient amplitude ($16 \pm 5\%$, $P<0.05$). This response was abolished in cells isolated from TPC2KO mice ($-6 \pm 4\%$, $P>0.05$).

NAADP photorelease in guinea pig atrial or ventricular myocytes caused a significant increase in calcium transient amplitude (of $37 \pm 8\%$ and $38 \pm 9\%$ respectively, both $P<0.05$), accompanied by acceleration in the rate of calcium transient decay (by $23 \pm 6\%$, atrial, and $28 \pm 9\%$, ventricular, myocytes, both $P<0.05$).

In the presence of KN93 (atrial myocytes) or AIP (ventricular myocytes), to inhibit cellular CaMKII function, no changes in Ca^{2+} transient amplitude or decay velocity were observed after NAADP photorelease ($P>0.05$, both measures, both cell types). Similarly, no changes in Ca^{2+} transients were observed during photorelease in the presence of the NAADP receptor antagonist, Ned-19 ($P>0.05$, both measures, both cell types).

These data support the hypothesis that NAADP-induced Ca^{2+} release requires TPC2, and suggest CaMKII is the major effector for its actions in cardiac myocytes.

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2. Macgregor et al. (2007) *J Biol Chem* 282: 15302.
3. Pitt et al. (2010) *J Biol Chem* 285: 35039.
4. Wang et al. (2012) *Cell* 151: 372.

1229-Plat

Calcium Signaling Inside Cilia Upon Mechanical Bending

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The primary cilium is a sensory organelle central to many signaling pathways. Direct visualization of signaling dynamics within primary cilia constitutes a major technical challenge due to the sub-micron dimensions of the organelle as well as its close proximity to the cell body. By newly designing and developing a genetically encoded calcium indicator (GECI) targeted to primary cilia without compromising indicator efficiency, we now demonstrate the unprecedented capability to visualize Ca^{2+} dynamics within the ciliary lumen with high specificity, sensitivity and wide dynamic range. Simultaneous

dual-color Ca^{2+} imaging at cytosol and primary cilia revealed highly resolved propagation of Ca^{2+} from the cytosol into distal tip of primary cilia in response to ATP. In addition, we provide direct evidence of dynamic Ca^{2+} signaling within primary cilia immediately after cilia bending caused by a mechanical flow. Therefore, the cilia-targeted GECI serves as a powerful tool to elucidating the roles of Ca^{2+} signaling in regulating sensory functions of primary cilia. The present approach is readily generalized to other signaling molecules and to other sub-micron cellular compartments.
(Reference: Su S. et al., Nature Methods, Accepted on 8/13/13, 10.1038/nmeth.2647).

Platform: Cell Mechanics and Motility II

1230-Plat
Mechanisms of Three-Dimensional Tumor Cell Motility in Dense Extracellular Matrices
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Tumor cell invasion requires navigation of tissue barriers of varying architecture. For instance, the extreme invasiveness of glioma cells is facilitated by their ability to migrate diffusely through brain parenchyma and approach guidance tracks along blood vessels. Brain parenchyma has a distinct physical structure characterized by densely packed neural cell processes and sub-micron extracellular space, and is largely devoid of the fibrillar collagen scaffolding typically found in stromal tissue. Consequently, glioma cells migrating in brain slices exhibit a distinct type of motility, with branched protrusions and hourglass-shaped cell-body deformations that help squeeze cells through tight spaces (Beadle et al., Mol. Biol. Cell 2008; 19(8):3357-68). However, the mechanistic details of this unique mode of motility remain incompletely understood. To address this question, we synthesized brain-mimetic nanoporous, non-fibrillar extracellular matrices (ECMs) based on cross-linked hyaluronic acid (HA), and verified that three-dimensional glioma cell motility in these ECMs strikingly recapitulated the phenotype seen in brain slices (Ananthanarayanan et al. Biomaterials 2011; 32(31):7913-23). Eliminating RGD peptides from the ECM abolished invasion, suggesting a requirement for integrin-mediated adhesions for this mode of motility. Increasing matrix density, or inhibiting myosin-based cellular contractility by blebbistatin or shRNA-induced knockdown of Myosin IIA severely impaired motility. We report preliminary results from two sets of studies: First, we investigate the balance of protrusive and contractile forces in glioma cell motility by pharmacologically and genetically manipulating the Rho GTPases RhoA, Rac1, and Cdc42. Second, we explore the involvement of actin-nucleating proteins such as Arp2/3 and formins in the protrusive dynamics observed in this mode of motility. Our studies help define the signaling mechanisms underlying the distinctive glioma cell motility observed in dense matrices.

1231-Plat
Transduction Channels' Gating Controls Friction on Vibrating Hair-Cell Bundles in the Ear
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Hearing starts when sound-evoked mechanical vibrations of the hair-cell bundle activate mechanosensitive ion channels, giving birth to an electrical signal. As for any mechanical machine, friction impedes movements of the hair bundle and thus constrains the sensitivity and frequency selectivity of auditory transduction. Viscous drag by the surrounding fluid on the micrometric hair-bundle structure provides a minimum source of friction. Using dynamic force measurements on single hair-cell bundles, we demonstrate here that the opening and closing of the transduction channels produce internal friction forces that can dominate hydrodynamic drag. A theoretical analysis reveals that channel friction arises from coupling the dynamics of the conformational change associated with channel gating to tip-link tension. In return, channel properties can control hair-bundle friction, with faster channels producing smaller friction. We propose that this intrinsic source of friction contributes to the process that sets the hair cell's characteristic frequency of responsiveness.

1232-Plat
Emergence of Collective Dynamics in Systems of Motile Cilia
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Motile cilia are fascinating structures, evolved very early in eukaryotes, and highly conserved throughout organisms of very different complexity. They generate the transport of fluid by periodic beating, through remarkably organized behaviour in space and time (e.g. collective waves). This allows simple unicellular organisms to swim, and allows transport of fluid in the airways and within the brain in humans.
It is not understood how these spatiotemporal patterns emerge, and what sets their properties. Individual cilia are nonequilibrium systems with many degrees of freedom. We have reduced these to fewer parameters, representing the effective force laws that drive oscillations, and paralleled with nonlinear phase oscillators studied in physics. At this level, the beating cilia become sources for a velocity field, which can be approximated (in the far field) by the Oseen tensor, or taking into account the presence of solid boundaries if necessary. This becomes a more tractable (albeit still non-linear and entirely not trivial) system on which to try and understand the emergence of collective dynamical states, and how the macroscopic characteristics are linked to the microscopic cilia parameters.
This presentation will report on insight gained by studying synthetic model phase oscillators, where colloidal particles are driven by optical traps (this keeps the length and time-scales of the living system, including the important role of thermal noise). The complex structural details of the cilia are coarse-grained into the details of how the colloidal particles are driven. We explore experimentally various colloidal models, finding in each case the conditions for optimal coupling. The applicability of this approach to biological data is illustrated by successfully mapping the behaviour of cilia in the alga Chlamydomonas onto the coarse-grained model, and linking the dynamics in a many-oscillator system to embryonic tissue development.

1233-Plat
Characterization of Different Dynamic Modes of a Crawling *Caenorhabditis Elegans* by Direct Measurement of Traction Force
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The traction force microscopy (TFM) is a technique widely used to measure cellular traction forces that are closely related to cell migration, mechanical signaling, and other cellular functions. We apply the TFM to characterize the dynamic force patterns in different crawling modes of *Caenorhabditis elegans* (*C. elegans*) on soft gel matrices of different stiffness. When *C. elegans* crawls forward, it concentrates the thrust force to localized regions along the body rather than forming a uniform load distribution in its lateral direction. The dynamic force distributions appeared differentially in different behavioral modes of *C. elegans* including the forward, backward movement, as well as a sharp turn called the Ω-turn. Such dynamic behaviors of *C. elegans* might be considered as an effort to minimize drag resistance by reducing contact area between its body and gel surface, and these observations are very similar to recent experimental study suggested for the slithering of snake on flat surface. This work was supported by the National Research Foundation (NRF) grant 2013-012420 (J. Park) and 2010-0016886 (S. Ahn and J. Shin).

1234-Plat
Optimality of Force Transmission in a Motor-Clutch Cellular Adhesion Model
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Microenvironmental mechanics play an important, but variable, role in determining cell morphology, traction, migration, proliferation, and differentiation with potential impacts on tumor development, growth, and invasion. Interestingly, some cell types have shown increasing migration and traction force as a function of substrate stiffness, while others have shown decreasing migration and traction force. These seemingly contradictory results may be explained by a motor-clutch model of cellular adhesion and force transmission which exhibits a maximum in traction force with respect to stiffness and may be tuned to different stiffness optima. Both stochastic and deterministic castings of the motor-clutch model provide a basis to explain the tuning of cells to different microenvironmental mechanics. A sensitivity analysis of the stochastic model suggests that molecular motors and adhesion clutches must approximately balance each other to achieve stiffness sensitivity. Consequently, individual parameters changes, which favor only the motors or the clutches, have little effect in shifting the stiffness optimum because the system loses stiffness sensitivity altogether. However, dual parameters changes, such that motors and clutches remain balanced, can shift the stiffness optimum over several orders of magnitude. This optimum occurs on the stiffness at which the time for all clutches to bind equals the cycle time of adhesion load and fail. At stiffnesses above this optimum, fewer than the maximum clutches bind, so the clutches are not utilized to their fullest extent. At stiffnesses below the optimum, clutches spontaneously fail at low loads because of the long cycle time, again resulting in an inefficient use of clutches. This determinant of the optimum stiffness was applied in conjunction with the deterministic motor-clutch model to derive a dimensionless quantity defining model behavior at any particular stiffness.